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(54) Title: ANTIBODIES WHICH BIND THE G-CSF RECEPTOR EXTRACELLULAR DOMAIN AND METHODS OF TREATMENT			
(57) Abstract <p>The present invention relates generally to cytokine interactive molecules, such as antibodies and other immune reactive molecules, agonists and antagonists. The present invention also provides methods for assaying for the presence of cytokines or receptor associated proteins such as kinases and their function.</p>			

ANTIBODIES WHICH BIND THE G-CSF RECEPTOR EXTRACELLULAR DOMAIN AND METHODS OF TREATMENT

FIELD OF THE INVENTION

5 The present invention relates generally to cytokine interactive molecules, such as antibodies and other immune reactive molecules, agonists and antagonists. The present invention also provides methods for assaying for the presence of cytokines or receptor associated proteins such as kinases and their function.

Throughout this specification, unless the context requires otherwise, the word 10 "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

BACKGROUND OF THE INVENTION

15 Gramulocyte colony-stimulating factor (G-CSF) stimulates the proliferation and differentiation of neutrophil precursors via interaction with a specific cell surface receptor, the G-CSF receptor (G-CSF-R).

Although the G-CSF-R has been cloned (1) and is functionally active in several different cell types (2), little is known about the mechanism of signal 20 transduction. The G-CSF-R is believed to consist of a single chain that is activated through ligand induced homodimerisation (3) as has been shown for the erythropoietin and growth hormone receptors (EPO-R, GH-R) (4). The G-CSF-R does not contain an intrinsic protein kinase domain (1) although tyrosine kinase activity seems to be required for transduction of the G-CSF signal (5). JAK kinases (6,7) are receptor-associated proteins which are rapidly phosphorylated after receptor activation. In particular, Tyk2 is phosphorylated following interferon α -receptor (IFN α -R) activation (8) and JAK2 following the binding of EPO (9), GH (10) and interleukin-3 (IL-3) (11) to their respective receptors.

In work leading up to the present invention, the inventors investigated early 30 signal transduction events resulting from the association of G-CSF with its receptor and the role of JAK1 and JAK2.

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In accordance with the present invention, antibodies were prepared to the extracellular domain of G-CSF-R. It has now been surprisingly discovered that G-CSF interaction with G-CSF-R is required for tyrosine phosphorylation of JAK kinases. The antibodies of the present invention now provide for a method of 5 inhibiting G-CSF binding to its receptor and, by consequence, phosphorylation by JAK kinases. The present invention contemplates, therefore, a method for treating G-CSF related disease conditions or JAK1 and JAK2 phosphorylation associated disease conditions which result from G-CSF interaction with its receptor.

10 SUMMARY OF THE INVENTION

One aspect of the present invention is directed to a composition comprising antibodies or parts, fragments or derivatives thereof to G-CSF-R extracellular domain.

Another aspect of the present invention relates to antibodies to the composition defined above.

15 Yet another aspect of the present invention contemplates a method for inhibiting, reducing or otherwise decreasing tyrosine phosphorylation of JAK1 or JAK2 in a mammal, said method comprising administering to said mammal a binding effective amount of an antibody or a part, fragment or derivative thereof interactive with G-CSF-R extracellular domain.

20 Still yet another aspect of the present invention contemplates a method for inhibiting, reducing or otherwise decreasing G-CSF interaction with G-CSF-R in a mammal, said method comprising administering to said mammal, a binding effective amount of an antibody or part, fragment or derivative thereof interactive with G-CSF-R extracellular domain.

25 In still yet another aspect of the present invention, there is provided agonists and antagonists to G-CSF-R.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a photographic representation showing tyrosine phosphorylation 30 of JAK kinases in response to G-CSF (AML-193 cells).

AML-193 cells were incubated with rhG-CSF (100 ng/ml) for the times indicated (minutes) and lysed. Tyrosine phosphorylated proteins were

Ac. neutralizantes

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were electrophoretically transferred to nitrocellulose and immunoblotted with either JAK2 antiserum (M7) (left panel) for JAK2 antiserum (UBI) (right panel).

Figure 5 is a photographic representation showing that different cytokines result in tyrosine phosphorylation of specific JAK kinases in AML-193 cells.

5 AML-193 cells were incubated with either G-CSF, GM-CSF or IL-6 at 100 ng/ml or medium (control) for 10 minutes and immunoprecipitated with antiphosphotyrosine antibody 4G10 (α PY). Immunoprecipitated proteins were separated by SDS/6% w/v PAGE and analysed by Western blot with either JAK1 antiserum (M7) (α JAK1), JAK2 antiserum (UBI) (α JAK2) or TYK2 antiserum (Santa 10 Crus) (α TYK2). The mobilities of the pre-stained molecular weight markers are 15 shown on the left.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention relates generally to antibodies to G-CSF-R. Preferably, 15 the antibodies are directed to the extracellular domain of G-CSF-R and more particularly a recombinant form of this molecule. The present invention is also directed to parts, fragments or derivatives of such antibodies which still interact with the G-CSF-R. The antibodies of the present invention are generally in isolated or purified form meaning that a composition comprises at least 25%, more preferably 20 at least 35%, even more preferably at least 45-50%, still more preferably at least 60-70% and even still more preferably at least 80-95% of the antibodies as determined by weight, immunoreactivity or other convenient means. Alternatively, the antibodies may be present in the form of isolated culture supernatant, tissue extract, serum or whole blood or ascites fluid.

25 Preferably, the G-CSF-R is of mammalian origin such as from a human, livestock animal (e.g. cow, horse, sheep, goat or donkey), laboratory test animal (e.g. mouse, rat or rabbit), companion animal (e.g. dog or cat) or captive wild animal (e.g. dingo, fox, wild boar or kangaroo). The most preferred receptors are of human and laboratory test animal origin (e.g. murine species).

30 Where the antibodies are polyclonal antibodies, they may be generated in any convenient host including a human, livestock animal, companion animal or captive wild animal as exemplified above. Where the antibodies are monoclonal antibodies,

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they may be prepared in any convenient hybridoma such as of murine (i.e. mouse or rat) origin.

The receptor used to generate the antibodies may be the whole receptor such as in purified, partially purified or isolated form including in the form of isolated membrane preparations. The receptor may also be produced by recombinant procedures or synthetic procedures or a combination thereof. In a particularly preferred embodiment, a fragment of the receptor is used which, in an even more preferred embodiment, is fused to a suitable carrier or marker molecule such as FLAG protein or alkaline phosphatase (AP). Glutathione-S-transferase (GST) may also be used.

According to this preferred embodiment, there is provided an antibody or part, fragment or derivative thereof interactive with a non-full length G-CSF-R fused to a carrier molecule. The non-full length portion of the receptor acts as a hapten and is or forms part of the extracellular domain. Preferably, the carrier molecule is FLAG or AP. Alternatively, the carrier molecules is GST.

The resulting fusion molecule may then be used to generate polyclonal or monoclonal antibodies which may undergo immunoabsorbent procedures to provide a composition of substantially, for example, extracellular domain-reactive receptor antibodies.

The terms "antibody" or "antibodies" are used herein in their broadest sense and include parts, fragments, derivatives, homologues or analogues thereof, peptide or non-peptide equivalents thereof and fusion molecules between two or more antibodies or between an antibody and another molecule. The antibodies or other immunointeractive molecules may also be in recombinant or synthetic form.

Accordingly, the present invention contemplates mutants and derivatives of the antibodies which interact with G-CSF-R extracellular domains. Mutants and derivatives of such antibodies include amino acid substitutions, deletions and/or additions. Furthermore, amino acids may be replaced by other amino acids having like properties, such as hydrophobicity, hydrophilicity, electronegativity, bulky side chains, interactive and/or functional groups and so on. Glycosylation variants and hybrid antibodies are also contemplated by the present invention.

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Amino acid substitutions are typically of single residues; insertions usually will be of the order of about 1-10 amino acid residues; and deletions will range from about 1-20 residues. Deletions or insertions preferably are made in adjacent pairs, i.e. a deletion of 2 residues or insertion of 2 residues.

5 The amino acid variants referred to above may readily be made using peptide synthetic techniques well known in the art, such as solid phase peptide synthesis and the like, or by recombinant DNA manipulations. Techniques for making substitution mutations at predetermined sites in DNA having known sequence are well known, for example through M13 mutagenesis. The manipulation of DNA sequences to produce 10 variant proteins which manifest as substitutional, insertional or deletional variants are well known in the art.

Other examples of recombinant or synthetic mutants and derivatives of the antibodies of the present invention include single or multiple substitutions, deletions and/or additions to any molecule associated with the ligand such as carbohydrates, 15 lipids and/or proteins or polypeptides. Naturally occurring or altered glycosylated forms of the subject antibodies are particularly contemplated by the present invention.

Amino acid alterations to the subject polypeptide contemplated herein include insertions such as amino acid and/or carboxyl terminal fusions as well as intra-sequence insertions of single or multiple amino acids. Generally, insertions within 20 the amino acid sequence will be smaller than amino or carboxyl terminal fusions, of the order of about 1 to 4 residues. Insertional amino acid sequence variants are those in which one or more amino acid residues are introduced into a predetermined site in the protein. Deletional variants are characterised by the removal of one or more amino acids from the sequence. Substitutional variants are those in which at least one 25 residue in the sequence has been removed and a different residue inserted in its place. Such substitutions may be made in accordance with Table 1.

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butylglycine, norvaline, phenylglycine, ornithine, sarcosine, 4-amino-3-hydroxy-6-methylheptanoic acid, 2-thienyl alanine and/or D-isomers of amino acids.

Crosslinkers can be used, for example, to stabilise 3D conformations, using homo-bifunctional crosslinkers such as the bifunctional imido esters having $(CH_2)_n$ 5 spacer groups with $n = 1$ to $n = 6$, glutaraldehyde, N-hydroxysuccinimide esters and hetero-bifunctional reagents which usually contain an amino-reactive moiety such as N-hydroxysuccinimide and another group specific-reactive moiety such as maleimido or dithio moiety (SH) or carbodiimide (COOH). In addition, peptides could be 10 conformationally constrained by, for example, incorporation of C_α and N_α-methylamino acids, introduction of double bonds between C_α and C_β atoms of amino acids and the formation of cyclic peptides or analogues by introducing covalent bonds such as forming an amide bond between the N and C termini, between two side chains or between a side chain and the N or C terminus.

The present invention, therefore, extends to amino acid and/or chemical 15 analogues of the subject antibodies having the identifying characteristics of being interactive with the extracellular domain of G-CSF-R.

Accordingly, reference herein to an antibody includes the naturally occurring molecule, recombinant, synthetic and analogue forms thereof and to any mutants, derivatives and human and non-human homologues thereof including amino acid and 20 glycosylation variants.

The antibodies of the present invention may be used to develop a new range of therapeutic and diagnostic agents. For example, the antibodies or fragments or derivatives thereof may act as antagonists and be useful, for example, in the treatment of G-CSF related disease conditions which result from G-CSF interaction with its 25 receptor, including JAK family phosphorylation related disorders (e.g. some cancers and tumours). They may also be used for screening for agonists useful, for example, where G-CSF interaction (or JAK1 or JAK2 phosphorylation) is to be promoted. Normal, abnormal or mutant receptor structure or receptor expression may also be determined through immunoreactivity studies.

30 According to this latter embodiment, there is contemplated a method of detecting a G-CSF-R on a cell in a biological sample, said method comprising contacting said sample with an antibody capable of binding to the extracellular domain

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of said G-CSF-R immobilised to a solid support for a time and under conditions sufficient for an antibody G-CSF-R complex to form and then detecting the presence of said complex.

In one preferred method, the complex is detected by contacting the complex 5 with a second antibody against the first mentioned antibody with the second antibody being labelled with a reporter molecule. Alternatively, the first antibody itself is labelled with a reporter molecule.

The first and second antibodies may be polyclonal or monoclonal and both are obtainable by immunisation of a suitable animal with the interactive molecule and 10 either type is utilisable in the assay. The methods of obtaining both types of sera are well known in the art. Polyclonal sera are less preferred but are relatively easily prepared by injection of a suitable laboratory animal with an effective amount of immunointeractive molecule, or antigenic parts thereof, collecting serum from the animal, and isolating specific antibodies by any of the known immunoabsorbent 15 techniques. Although antibodies produced by this method are utilisable in virtually any type of assay, they are generally less favoured because of the potential heterogeneity of the product.

The use of monoclonal antibodies in the above immunoassay is particularly preferred because of the ability to produce them in large quantities and the 20 homogeneity of the product. The preparation of hybridoma cell lines for monoclonal antibody production derived by fusing an immortal cell line and lymphocytes sensitized against the immunogenic preparation can be done by techniques which are well known to those who are skilled in the art. (See, for example Douillard and Hoffman, *Basic Facts about Hybridomas*, in *Compendium of Immunology* Vol II, ed. 25 by Schwartz, 1981; Kohler and Milstein, *Nature* 256: 495-499, 1975; *European Journal of Immunology* 6: 511-519, 1976).

The presence of a G-CSF-R may be accomplished in a number of ways such as by Western blotting and ELISA procedures. A wide range of immunoassay techniques are available as can be seen by reference to US Patent Nos. 4,016,043, 30 4,424,279 and 4,018,633. These, of course, include both single-site and two-site or "sandwich" assays of the non-competitive types, as well as in the traditional

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competitive binding assays. These assays also include direct binding of a labelled antibody to a target.

Sandwich assays are among the most useful and commonly used assays and are particularly useful in the present invention. A number of variations of the 5 sandwich assay technique exist, and all are intended to be encompassed by the present invention. Briefly, in a typical forward assay, an antibody is brought into contact with a biological sample comprising cells potentially carrying G-CSF-R. After a suitable period of incubation, for a period of time sufficient to allow formation of an antibody G-CSF-R complex, a second antibody specific to the complexed antibody, 10 labelled with a reporter molecule capable of producing a detectable signal, is then added and incubated allowing sufficient time for the formation of a tertiary complex. Any unreacted material is washed away, and the presence of the second antibody bound to the first antibody is determined by observation of a signal produced by the reporter molecule. The results may either be qualitative, by simple observation of 15 the visible signal, or may be quantitated by comparing with a control sample containing known amounts of hapten. Variations on the forward assay include using a first antibody (against G-CSF-R) labelled with a reporter molecule. In addition, the antibodies or cells carrying G-CSF-R may be immobilised onto a solid support.

Suitable solid supports include glass or a polymer, the most commonly used 20 polymers being cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene. The solid supports may be in the form of tubes, beads, discs of microplates, or any other surface suitable for conducting an immunoassay. The binding processes are well-known in the art and generally consist of cross-linking covalently binding or physically adsorbing molecules to the polymer.

25 "Reporter molecule", as used in the present specification, is meant a molecule which, by its chemical nature, provides an analytically identifiable signal which allows the detection of antigen-bound antibody. Detection may be either qualitative or quantitative. The most commonly used reporter molecules in this type of assay are either enzymes, fluorophores or radionuclide containing molecules (i.e. radioisotopes) 30 and chemiluminescent molecules.

In the case of an enzyme immunoassay, an enzyme is conjugated to the immunointeractive molecule or an antibody thereto generally by means of

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glutaraldehyde or periodate. As will be readily recognised, however, a wide variety of different conjugation techniques exist, which are readily available to the skilled artisan. Commonly used enzymes include horseradish peroxidase, glucose oxidase, beta-galactosidase and alkaline phosphatase, amongst others. The substrates to be used with the specific enzymes are generally chosen for the production, upon hydrolysis by the corresponding enzyme, of a detectable colour change. Examples of suitable enzymes include alkaline phosphatase and peroxidase. It is also possible to employ fluorogenic substrates which yield a fluorescent product rather than the chromogenic substrates noted above. Generally, the enzyme-labelled antibody is added to the immunointeractive molecule-receptor complex, allowed to bind, and then the excess reagent is washed away. Alternatively, an enzyme labelled immunointeractive molecule is used. A solution containing the appropriate substrate is then added to the tertiary complex. The substrate will react with the enzyme linked to the antibody/immunointeractive molecule, giving a qualitative visual signal, which may be further quantitated, usually spectrophotometrically, to give an indication of the amount of hapten which was present in the sample. "Reporter molecule" also extends to use of cell agglutination or inhibition of agglutination such as red blood cells on latex beads, and the like.

Alternately, fluorescent compounds, such as fluorescein and rhodamine, may be chemically coupled to antibodies without altering their binding capacity. When activated by illumination with light of a particular wavelength, the fluorochrome-labelled antibody absorbs the light energy, inducing a state of excitability in the molecule, followed by emission of the light at a characteristic colour visually detectable with a light microscope. Immunofluorescence and EIA techniques are both very well established in the art and are particularly useful for the present method. However, other reporter molecules, such as radioisotope, chemiluminescent or bioluminescent molecules, may also be employed.

By detecting G-CSF-R by the procedures, aberrant receptors may be discerned thus providing a useful screening procedure for potential disease conditions.

The present invention also provides a pharmaceutical composition comprising an effective amount of antibodies capable of binding or otherwise associating with the extracellular domain of G-CSF-R and one or more pharmaceutically acceptable

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carriers and/or diluents. The active ingredients of a pharmaceutical composition comprising the antibodies are contemplated to exhibit excellent therapeutic activity, for example, in the treatment of G-CSF disease conditions such as some cancers, in an amount which depends on the particular case. For example, from about 0.5 μ g 5 to about 20 mg per kilogram of body weight per day of antibody may be administered. Dosage regima may be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation.

10 The active compound may be administered in a convenient manner such as by the oral, intravenous (where water soluble), intramuscular, subcutaneous, intranasal, intradermal or suppository routes or implanting (eg using slow release molecules). Depending on the route of administration, the active ingredients which comprise the immunointeractive molecules may be required to be coated in a material to protect 15 said ingredients from the action of enzymes, acids and other natural conditions which may inactivate said ingredients. In order to administer the immunointeractive molecules by other than parenteral administration, they will be coated by, or administered with, a material to prevent its inactivation. For example, the immunointeractive molecules may be administered in an adjuvant, co-administered 20 with enzyme inhibitors or in liposomes. Adjuvant is used in its broadest sense and includes any immune stimulating compound such as interferon. Adjuvants contemplated herein include resorcinols, non-ionic surfactants such as polyoxyethylene oleyl ether and n-hexadecyl polyethylene ether. Enzyme inhibitors include pancreatic trypain inhibitor, diisopropylfluorophosphate (DEP) and trasylo. Liposomes include 25 water-in-oil-in-water emulsions as well as conventional liposomes.

30 The active compounds may also be administered parenterally or intraperitoneally. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the

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enable detection of receptor-associated proteins. Polyclonal rabbit antiserum, designated R55, was generated by immunisation with a pGEX/G-CSF-R bacterial fusion protein encompassing the haemopoietin receptor domain. A BamH1 fragment of the receptor cDNA encoding amino acids 17-345 was inserted into the BamH1 site 5 of PGEX-1 (13). The monoclonal antibody to the G-CSF-R, LMM174 was raised against CHO-6A11 cells.

The monoclonal antibody LMM741 and rabbit serum R55 detected the receptor in cell lysates from CHO-6A11 and the human monocytic leukaemic cell line AML-193 (14) (Figure 1A). Two protein of apparent molecular mass 130 and 110 10 kDa were detected in CHO-6A11 lysates (Figure 1A, lane D) and were not present in untransfected CHO-K1 cell lysates (Figure 1A, lane C) or when non-immune rabbit serum was used (Figure 1A, lanes A and B). Three proteins of apparent molecule mass 145, 135 and 115 kDa were detected in AML-193 cells (Figure 1A, lane E). The different sized proteins observed are likely to be due to differently glycosylated 15 forms of the receptor (2). AML-193 cells proliferated in response to G-CSF whereas CHO-6A11 cells did not (Figure 1B).

Stimulation of CHO-6A11 cells with G-CSF induced a tyrosine phosphorylated band of 130 kDa which was immunoprecipitated by antiserum to JAK1 (M7) (Figure 2A). M7 was generated by immunisation with a pGEX/JAK1 bacterial fusion protein 20 spanning the first kinase-like domain of JAK1 (amino acids 576-825) (8). When compared with JAK2 antiserum, M7 detects a protein which migrates slower on SDS-PAGE than JAK2, suggesting that it is specific for JAK1, despite the close homology of domain 1 between JAK1 and JAK2 (15).

Phosphorylation of JAK1 was also observed by immunoprecipitation with M7 25 and immunoblotting with anti-phosphotyrosine (Figure 2B). When this blot was stripped and re-probed with M7, the result (Figure 2B, lower panel), showed that there was equal sample loading in all lanes. Tyrosine phosphorylation of JAK1 was observed after 2 minutes of G-CSF stimulation, was maximal between 10 and 20 minutes and still evident, although decreasing, after 60 minutes.

Tyrosine phosphorylation of JAK1 in response to G-CSF was also observed 30 in the AML-193 cells (Figure 2C). Increased levels of phosphotyrosine have proved to be an excellent index of the involvement of particular protein tyrosine kinases in

signal transduction pathways (15). The involvement of JAK1 in intracellular events triggered by G-CSF was thus strongly indicated by these data.

An *in vitro* kinase assay (11) was used to test whether the increased tyrosine phosphorylation of JAK1 correlated with an increase in intrinsic kinase activity.

5 When incubated with [γ -³²P]ATP, JAK1 showed an increased capacity to autophosphorylate in response to G-CSF stimulation (Figure 3A). A higher molecular weight band of unknown identity was also phosphorylated. The kinase activity appeared to be maximal at 30 minutes. Phosphoamino acid analysis from kinase assay samples of 0 and 30 minutes after G-CSF stimulation confirmed that JAK1 was 10 phosphorylated on tyrosine residues (Figure 3B). Some increase in phosphorylation on serine residues was also observed. This is in contrast to the observation that JAK2 incorporates ³²P into tyrosine and threonine residues (9). Thus, the elevated kinase activity of JAK1 upon activation of CHO-6A11 cells by G-CSF underscores the likely involvement of this molecule in G-CSF-mediated signal transduction.

15 To determine whether JAK1 is physically associated with the G-CSF-R, a receptor immunoprecipitate was analysed by blotting with M7. JAK1 co-immunoprecipitated with the G-CSF-R both before and after G-CSF treatment of CHO-6A11 cells (Figure 4, lanes A and B). This band was not observed when G-CSF-R immunoprecipitates were blotted with M7 pre-immune serum. The amount 20 of JAK1 co-immunoprecipitate remained constant for at least 20 minutes (not shown) and appeared to be a relatively small percentage of total cytoplasmic JAK1 (Figure 4, lanes C and D). Receptor immunoprecipitates also showed *in vitro* kinase activity corresponding to a 130 kDa protein.

Stimulation of CHO-6A11 cells with G-CSF also resulted in phosphorylation 25 of the G-CSF-R on tyrosine within 2 minutes of G-CSF stimulation (Figure 5). The phosphorylated receptor migrated at a higher apparent molecular mass (150 and 135 kDa) than the two main bands detected by LMM741 and R55. Differential migration of phosphorylated protein has been observed with other proteins, for example, STAT91 (16) and middle sized tumour antigen (17). Phosphorylation of the murine 30 G-CSF-R in a transfected hemopoietic cell line (32D) has also been detected recently by Pan *et al.* (18).

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The G-CSF-R shows considerable homology with gp130 (46.3% amino acid similarity) (2), signal transducing component of the IL-6, oncostatin M, LIF, CNTF and IL-11 receptors. Of interest, two of the three highly conserved regions of homology between gp-130 and the G-CSF-R have been shown to be necessary for 5 signal transduction, most notably a 99 amino acid region of the cytoplasmic domain (2). Consistent with the notion that this homology indicates similar function, it has been recently observed JAK1 association with gp-130 following IL-6 and LIF stimulation.

The data presented here are strongly suggestive of an important role for JAK1 10 in G-CSF receptor signal transduction pathways. The co-precipitation of G-CSF and JAK1 demonstrates a close association between these molecules. Furthermore, phosphorylation of both within two minutes of G-CSF binding makes it likely that JAK1 is the kinase responsible for G-CSF-R phosphorylation. Recent reports indicate that JAK1 is interdependent with at least one other JAK family kinase in signalling 15 pathways for IFN α and IFN γ receptors.

Although the CHO-6A11 system is an artificial one, it is clear that AML-193 cells, which proliferate in response to G-CSF, share the same JAK1 response (Figure 2C). This suggests that these observations can also be applied to signal transduction from the native G-CSF-R.

20 Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and 25 all combinations of any two or more of said steps or features.

CLAIMS:

1. A composition comprising antibodies or parts, fragments or derivatives thereof to G-CSF receptor extracellular domain.
2. The composition of claim 1 wherein the G-CSF receptor extracellular domain is in recombinant form.
3. The composition of claim 1 or claim 2 wherein the antibodies are polyclonal antibodies.
4. The composition of claim 1 or claim 2 wherein the antibodies are monoclonal antibodies.
5. The composition of claim 1 or claim 2 wherein the antibodies are labelled with a reporter molecule capable of providing an identifiable signal.
6. An antibody to the composition of antibodies defined in claim 1.
7. An antibody according to claim 6 labelled with a reporter molecule capable of providing an identifiable signal.
8. A method for inhibiting or decreasing tyrosine phosphorylation of a JAK kinase in a mammal comprising administering to said mammal a binding effective amount of an antibody or a part, fragment or derivative thereof interactive with G-CSF receptor extracellular domain.
9. The method of claim 8 wherein the antibody is specific for a recombinant form of the G-CSF receptor extracellular domain.
10. The method of claim 8 or claim 9 wherein the antibody is a polyclonal antibody.
11. The method of claim 8 or claim 9 wherein the antibody is a monoclonal antibody.
12. The method of claim 8 wherein the mammal is a human, livestock animal, companion animal or laboratory test animal.
13. The method of claim 12 wherein the mammal is a human or murine species.
14. A method for inhibiting or decreasing G-CSF interaction with G-CSF receptor in a mammal comprising administering to said mammal a binding effective amount of an antibody or part, fragment or derivative thereof interactive with G-CSF receptor extracellular domain.

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15. The method of claim 14 wherein the antibody is directed against a recombinant form of G-CSF receptor extracellular domain.
16. The method of claim 14 or claim 15 wherein the antibody is a polyclonal antibody.
17. The method of claim 14 or claim 15 wherein the antibody is a monoclonal antibody.
18. The method of claim 14 wherein the mammal is a human, livestock animal, companion animal or laboratory test animal.
19. The method of claim 19 wherein the mammal is a human or murine species.
20. An antagonist to G-CSF receptor.
21. The antagonist of claim 20 wherein said antagonist is an antibody or part, fragment or derivative thereof directed against G-CSF receptor extracellular domain.
22. The antagonist of claim 21 wherein the antibody is directed against G-CSF receptor extracellular domain in recombinant form.
23. The antagonist of claim 21 or claim 22 wherein said antagonist is a polyclonal antibody.
24. The antagonist of claim 21 or claim 22 wherein said antagonist is a monoclonal antibody.
25. A method of treating a condition resulting from G-CSF interaction with its receptor comprising administering a pharmaceutically effective amount of antibody specific for G-CSF receptor extracellular domain.
26. The method of claim 25 wherein the antibody is directed against a recombinant form of G-CSF receptor extracellular domain.
27. The method of claim 25 or claim 26 wherein the antibody is a polyclonal antibody.
28. The method of claim 25 or claim 26 wherein the antibody is a monoclonal antibody.
29. The method of claim 25 wherein the mammal is a human, livestock animal, companion animal or laboratory test animal.